METHODS FOR MODULATION OF OOCYTE ACTIVATION

BACKGROUND OF THE INVENTION

In mammals, life begins at fertilization when the sperm interacts with the oocyte to trigger a series of intracellular Ca^{2+} oscillations that persists for several hours. This prolonged period of repetitive Ca^{2+} pulses triggers the developmental program by stimulating the enzymatic machinery involved in the cell division cycle. Further changes in intracellular Ca^{2+} are not observed until the one cell embryo is ready to divide, when a spontaneous Ca^{2+} transient triggers cleavage to form two daughter cells. The early steps that lead to the rises in calcium and oocyte activation at fertilization were unknown but of great interest, particularly with the advent of *in vitro* fertilization techniques, and whole-animal cloning by nuclear transfer. This calcium rise is required for oocyte activation and the subsequent events of development in eggs or oocytes of all species.

The meiotic division of mammalian oocytes begins with one primary germ cell (oocyte), which gives rise to only one mature ovum (egg). In normal mammalian development, oocytes become developmentally arrested in the ovaries at the germinal vesicle stage in prophase of the first meiotic division. Upon appropriate stimulation, meiosis resumes, the germinal vesicle breaks down, and the first meiotic division is completed with the extrusion of a diploid set of chromosomes into the first polar body, another diploid set of chromosomes remaining within the cytoplasm of the oocyte. The oocyte then becomes arrested at metaphase of the second meiosis ("Met II"). Met II oocytes (mature oocytes) can then be ovulated and fertilized.

Once fertilized, the activated oocyte completes the second meiotic division with the extrusion of a haploid set of chromosomes into the second polar body, male and female pronuclei are formed, and DNA replication is initiated in the pronuclei. The male and female pronuclei then fuse together, allowing their chromosomes to mingle. Equal segregation of the genetic material occurs by mitosis and the zygote cleaves to form two daughter blastomeres. The embryo continues to develop by undergoing a series of mitotic divisions before differentiating into specific cells, resulting in the organization of tissues and organs. This developmental program ensures the successful transition from oocyte to offspring.

When this process is defective, for example the inability of sperm to fertilize the egg, technical assistance has been sought. Since the first application of *in vitro* fertilization (IVF) in humans, the number of patients using assisted reproductive technologies (ART) has increased tremendously in number and technological spectrum. Infertility affects approximately one out of every six couples in the United States who desir children. For about 30% of couples male